PCT/JP2005/003744

11.3.2005

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APPLICATION NUMBER: ***60/549,561***
FILING DATE: ***March 04, 2004***

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The Regulators of Expression of Adiponectin Receptors

Summary

Adiponectin/Acrp30 is a hormone secreted by adipocytes that acts as an antidiabetic and anti-atherogenic adipokine. We reported that AdipoR1/R2 serve as receptors for adiponectin and mediate increased fatty-acid oxidation and glucose uptake by adiponectin. In this study, we studied expression levels and roles of AdipoR1/R2 in several physiological and pathophysiological states such as fasting/refeeding, obesity and insulin resistance. Here we show that the expressions of AdipoR1/R2 in insulin target organs such as skeletal muscle and liver are significantly increased in fasted mice and decreased in refed mice. The expression of AdipoR1/R2 appears to be inversely correlated with plasma insulin levels *in vivo*. Indeed, STZ increased and insulin reduced the expression of AdipoR1/R2 *in vivo*. Interestingly, incubation of hepatocytes or myocytes with insulin reduced the expression of AdipoR1/R2 via PI3-kinase/Foxo1 dependent pathway *in vitro*. Moreover, the expressions of AdipoR1/R2 of ob/ob mice are significantly decreased in skeletal muscle and adipose tissue, which were also correlated with decreased adiponectin binding to membrane fractions of skeletal muscle and decreased AMP kinase activation by adiponectin. This subsequent decrease in adiponectin effects called "adiponectin resistance" in turn may play a role in worsing insulin resistance in ob/ob mice. In conclusion, the expression of AdipoR1/R2 appears to be regulated by several physiological and pathophysiological states such as fasting/refeeding and hyperinsulinemia via insulin/PI3-kinase/Foxo1 pathway, and correlated with adiponectin sensitivity.

Introduction

Adiponectin /Acrp30 (1-4) is a hormone secreted by adipocytes that acts as an antidiabetic(5-12) and anti-atherogenic(8,12,13) adipokine. This insulin sensitizing effect of adiponectin appears to be mediated by an increase in fatty acid oxidation via activation of the 5'-AMP-activated protein kinase (AMPK) (10,11) and peroxisome proliferator-activated receptor (PPAR)α (5,6,12). Very recently, we have reported the cloning of complementary DNAs encoding adiponectin receptors (AdipoR) 1 and 2 by expression cloning (14). AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver. AdipoR1 and R2 are predicted to contain seven transmembrane domains (14), but to be structurally and functionally distinct from G-protein coupled receptors (15-17). AdipoR1 and R2 serve as receptors for globular and full-length adiponectin, and mediate increased AMPK (10,11), PPARα ligands activities (12), and the fatty-acid oxidation and glucose uptake by adiponectin (14).

It has not been shown previously whether the expressions of AdipoR1 and R2 are altered in physiological and pathophysiological states or whether they are correlated with adiponectin sensitivity. To address these questions, we first studied the expressions of AdipoR1 and R2 during fasting and refeeding, and showed that the expression of AdipoR1/R2 is altered dramatically during fasting and refeeding of mice, closely paralleling the regulation of plasma insulin levels. The expression of AdipoR1/R2 appears to be inversely correlated with plasma insulin levels *in vivo*. These data identify AdipoR1/R2 as an important regulator of metabolism sensitive to nutritional status and insulin.

In the current studies, we next have analyzed the effect of insulin on the levels of the AdipoR1/R2 mRNAs in livers, skeletal muscles and adipose tissues of living mice. For this purpose, we treated mice with streptozotocin (STZ), which destroys the β-cells

of the pancreas and leads to an acute insulin deficiency (18). We show that this treatment leads to a profound increase in AdipoR1/R2 mRNA, and this is restored by administration of insulin. These data support an *in vivo* role for insulin in specifically repressing transcription driven by the AdipoR1/R2 promoter.

Moreover, we show here that insulin reduces AdipoR1/R2 mRNA in myocytes and hepatocytes. We next addresses the mechanism of insulin signalling for reduction of AdipoR1/R2 gene expression in myocytes. Interestingly, insulin incubation with insulin reduced the expression levels of AdipoR1/R2 via PI3-kinase/Foxo1 pathway (19) *in vitro*.

We also studied these expressions in models of altered insulin sensitivity such as an obesity-linked insulin resistance model. The expressions of AdipoR1/R2 of ob/ob mice are significantly decreased in muscle and adipose tissue, which were also correlated with decreased adiponectin binding to membrane fractions of these tissues and decreased AMP kinase activation by adiponectin. The subsequent decrease in adiponectin effects called "adiponectin resistance" in turn may play a role in worsing insulin resistance in ob/ob mice.

In conclusion, the expression of AdipoR1/ R2 appears to be regulated by several physiological and pathophysiological states such as fasting/refeeding and hyperinsulinemia, and correlated with adiponectin sensitivity. Our data also suggested that not only agonism of AdipoR1/R2 but also the strategies to increase AdipoR1/R2 may be a logical approach to provide a novel treatment modality for insulin resistance and type 2 diabetes.

Experimental Procedures

Chemicals All materials were from the sources given in the References (6, 10, 14 and 20).

Animals Fifteen-week-old ob/ob mice and their wild-type C57BL/6 mice were obtained from CharlesRiver Breeding Laboratories (Wilmington, MA). Male mice were housed in colony cages, maintained on a 12-h light/12-h dark cycle. Our high-fat diet contains oil 1152g (from Benibana, Japan; safflower oil [high-oleic type] contained 46% oleic acid (18:1n-9) and 45% linoleic acid (18:2n-6) from total fatty acids), casein 1191.6g (Oriental Yeast, Tokyo, Japan, No.19), sucrose 633.6g (Oriental Yeast, No. 13), vitamin mix 50.4g (Oriental Yeast, No.20 (AIN76), mineral mix 352.8g (Oriental Yeast, No.25 (AIN76), cellulose powder 201.6g (Oriental Yeast, No.19), DL-methionine 18g (Wako Pure Chemicals, Osaka, Japan), water 360 ml; total 3600 g (20). Plasma insulin was measured by an insulin immunoassay (Shibayagi, Gunma, Japan). The animal care and procedures were approved by the Animal Care Committee of the University of Tokyo.

STZ Treatments Diabetes was induced by a double intraperitoneal injection of 0.2-0.3 ml of 50 mM sodium citrate solution (pH 4.5) containing STZ (200 mg/kg body weight). Control (non-STZ) mice were injected with 50 mM sodium citrate solution (pH 4.5). Three days after injection, plasma glucose levels were checked and diabetes was confirmed (glucose level >250 mg/dl). A combination of human regular insulin (1units/kg; Eli Lilly, Indianapolis, IN), each given in 0.2 ml of saline was administered to the STZ+insulin group. The mice in the STZ+vehicle and non-STZ groups received 0.2 ml of saline injected both intraperitoneally . After injection of INS or saline, the animals were fasted for 6 h and then hindlim's skeletal muscles were removed.